Epitect Bisulfite Kit Protocol (Qiagen)

Bisulfite DNA Converstion

1. Thaw DNA. Disolve required number of aliquots of Bisulfite Mix by adding 800 µl RNase free water to each aliquot . Vortex until completely dissolved (up to 5 min).
2. Prepare bisulfite reactions in 200 µl PCR tubes according to below. Add in order listed for total volume of 140 µl
    a. 1 ng – 2 µg DNA solution
    b. RNase-free water to 20 µl total (DNA + Water must be 20 µl)
    c. 85 µl Bisulfite mix (dissolved)
    d. 35 µl DNA Protect Buffer
3. Close PCR tubes and mix bisulfite reactions thoroughly. Store Tubes at room temperature. Note: DNA protect buffer should turn from green to blue after addition to DNA-Bisulfite mix indicating sufficient mixing and correct pH.
4. Program a thermal cycler with the Bisulfite conversion conditions (GEMBI program)
5. Split each tube into 3 different tubes (so volumes are under 50µl). Place the PCR tubes containing the bisulfite reaction into thermal cycler and start program.

Clean up of bisulfite converted DNA

6. Once bisulfite conversion is complete, centrifuge PCR tubes containing bisulfite reactions briefly, then transfer the complete bisulfite reactions to clean 1.5ml microcentrifuge tubes.
7. Add 560µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA. Mix solution by vortexing and centrifuge briefly. (Carrier RNA is not necessary when using greater than 100 ng DNA)
8. Place an EpiTect Spin Column and collection tube in a suitable rack. Transfer the whole mixture from step 7 into EpiTect spin columns.
9. Centrifuge columns at max speed for 1 minute. Discard flow-through and place spin column back in collection tube.
10. Add 500 µl Buffer BW to the spin column and centrifuge at max speed for 1 min. Discard flow-through and place spin column back into collection tube.
11. Add 500 µl Buffer BD (desulfonation buffer) to spin column and incubate for 15 min at room temperature.
12. Centrifuge column at max speed for 1 min. Discard flow-through and place column back into collection tube.
13. Add 500 µl Buffer BW and centrifuge at max speed for 1 min. Discard and replace spin column
14. Repeat step 13
15. Place spin column into new 2 ml collection tube and centrifuge at max speed for 1 min to remove residual liquid. (extend time to 5 minutes if used in real time PCR)
16. Place spin column into clean 1.5 ml microcentrifuge tube. Add 20 µl Buffer EB to center of membrane. Elute purified DNA by centrifugation for 1 min at approximately 15,000 x g. Store DNA at -20°C